Access DB# ____

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Gailene R Gabel Examiner # : 76197 Date: 9-16-12
Art Unit: 1641 Phone Number 305-1807 Serial Number: 09/311,176
Mail Box and Bldg/Room Location: 9D16 1641 Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: New Assay

Inventors (please provide full names): DeSena Smith, Dwarakanath, Srikian I.

Earliest Priority Filing Date: May 15, 1998

**For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

Please search claims 1, 2, 3 and highlighted terms

* TDP — stands for ... 5'-diphosphate

* divalent metal ion chelator = EDTA (claim 8) in preferred ...

* bacterial cell membrane = gram positive gram (-) bacteria (claim 4)

Point of Contact:
Beverly Shears
Technical Info. Specialist
CM1 12C14 Tel: 308-4994

thanks (!. Gail

****

STAFF USE ONLY	Type of Search	Vendors and cost where applicable
Searcher:	NA Sequence (#)	STN
Searcher Phone #:	AA Sequence (#)	Dialog
Searcher Location:	Structure (#)	Questel/Orbit
Date Searcher Picked Up:	Bibliographic	Dr.Link
Date Completed: 07-21-00	Litigation	Lexis/Nexis
Searcher Prep & Review Time: 12	Fulltext	Sequence Systems
Clerical Prep Time:	Patent Family	WWW/Internet
Online Time: 21	Other	Other (specify)

PTO-1590 (1-2000)

Gabel
09/341196

Specif. assay

```
     FILE 'CAPLUS' ENTERED AT 16:26:01 ON 21 JUL 2000
L1          247 S SPA(S)SCINTILL? OR SCINTILLAT?(1W)ASSAY?

     FILE 'REGISTRY' ENTERED AT 16:26:28 ON 21 JUL 2000
               E PEPTIDOGLYCAN/CN

     FILE 'CAPLUS' ENTERED AT 16:26:49 ON 21 JUL 2000
L2            2 S L1 AND (PEPTIDOGLYCAN OR PEPTIDO GLYCAN)

=> d 1-2 .bevstr1

L2   ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2000 ACS
ACCESSION NUMBER:         1999:753373  CAPLUS
DOCUMENT NUMBER:          132:1807
TITLE:                    A scintillation proximity
                          assay for the detection of
                          peptidoglycan synthesis
INVENTOR(S):              Desousa, Sunita; Prahlad, Dwarakanath
PATENT ASSIGNEE(S):       Astra Aktiebolag, Swed.
SOURCE:                   PCT Int. Appl., 20 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE          APPLICATION NO.  DATE
     ---------------   ----  --------      ---------------  --------
     WO 9960155         A1   19991125      WO 1999-SE749    19990504
         W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU,
             CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,
             IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,
             MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG,
             SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW,
             AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE,
             DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
             CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
     AU 9944029         A1   19991206      AU 1999-44029    19990504
PRIORITY APPLN. INFO.:                    IN 1998-MA1019   19980515
                                          SE 1998-2210     19980622
                                          WO 1999-SE749    19990504
AB   The invention provides a scintillation proximity
     assay for detecting peptidoglycan synthesis. The
     assay is esp. suitable for high throughput screening of compds.
     affecting peptidoglycan synthesis.
IT   Chelating agents
        (Divalent metal ion; a scintillation proximity
     assay for detection of peptidoglycan synthesis)
                         Searcher   :    Shears   308-4994
```

IT Radiochemical analysis
(**Scintillation** proximity **assay**; a **scintillation** proximity **assay** for detection of **peptidoglycan** synthesis)
IT Bacteria (Eubacteria)
Cell membrane
Drug screening
Escherichia coli
Fluorescent substances
Synthesis
(a **scintillation** proximity **assay** for detection of **peptidoglycan** synthesis)
IT **Peptidoglycans**
RL: BPN (Biosynthetic preparation); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation)
(a **scintillation** proximity **assay** for detection of **peptidoglycan** synthesis)
IT Transport proteins
RL: CAT (Catalyst use); USES (Uses)
(a **scintillation** proximity **assay** for detection of **peptidoglycan** synthesis)
IT Agglutinins and Lectins
RL: NUU (Nonbiological use, unclassified); USES (Uses)
(a **scintillation** proximity **assay** for detection of **peptidoglycan** synthesis)
IT Enzymes, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(antagonists; a **scintillation** proximity **assay** for detection of **peptidoglycan** synthesis)
IT Cations
(divalent; a **scintillation** proximity **assay** for detection of **peptidoglycan** synthesis)
IT Wheat
(germ, agglutinin; a **scintillation** proximity **assay** for detection of **peptidoglycan** synthesis)
IT Peptides, reactions
RL: RCT (Reactant)
(pentapeptides, UDP-N-acetylmuramyl; a **scintillation** proximity **assay** for detection of **peptidoglycan** synthesis)
IT 9033-07-2, Transglycosylase 9047-61-4, Transferase 9059-29-4, Transpeptidase 68858-66-2, Pyrophosphorylase
RL: CAT (Catalyst use); USES (Uses)
(a **scintillation** proximity **assay** for detection of **peptidoglycan** synthesis)
IT 60-00-4, Edta, uses
RL: NUU (Nonbiological use, unclassified); USES (Uses)
(a **scintillation** proximity **assay** for

detection of **peptidoglycan** synthesis)

IT 528-04-1D, radiolabeled 25126-51-6, Undecaprenyl phosphate 251294-78-7
RL: RCT (Reactant)
(a **scintillation** proximity **assay** for detection of **peptidoglycan** synthesis)

REFERENCE COUNT: 2
REFERENCE(S): (1) Amersham International PLC; WO 9426413 A1 1994 CAPLUS
(2) Cook, N; Drug discovery today 1996, V1(7), P287 CAPLUS

L2 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1998:534347 CAPLUS
DOCUMENT NUMBER: 129:260832
TITLE: Synthesis of a radioiodinated Park nucleotide analog: a new tool for antibacterial screen development
AUTHOR(S): Eid, Clark N.; Nesler, Michael J.; Zia-Ebrahimi, Mohammad; Wu, Chuyn-Yeh Ernie; Yao, Raymond; Cox, Karen; Richardson, John
CORPORATE SOURCE: Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN, 46285, USA
SOURCE: J. Labelled Compd. Radiopharm. (1998), 41(8), 705-716
CODEN: JLCRD4; ISSN: 0362-4803
PUBLISHER: John Wiley & Sons Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The Park nucleotide is an important biol. building block used in the construction of bacterial cell walls. Herein, the authors describe the synthesis of radiolabeled Park nucleotide analog p-125I-C6H4OCH2CO-Ala-D-Glu(Lys-D-Ala-D-Ala-OH)-OH, wherein the uridine diphosphate-N-acetylmuramyl moiety has been replaced with an iodobenzene isostere, using electrophilic destannylation. Anti-Park nucleotide antibody binding assays using a **scintillation** proximity **assay** (**SPA**) system showed good recognition of the radiolabeled surrogate. This methodol. could be used for establishing a screen to identify inhibitors of **peptidoglycan** biosynthesis.

IT Antibodies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(prepn. of radioiodinated Park nucleotide analog as new tool for antibacterial screen development)

IT 18836-50-5DP, Park nucleotide, radiolabeled iodophenyl analog 213532-14-0P 213532-23-1P
RL: BAC (Biological activity or effector, except adverse); SPN

(Synthetic preparation); BIOL (Biological study); PREP (Preparation)
(prepn. of radioiodinated Park nucleotide analog as new tool for antibacterial screen development)

IT 1878-94-0, p-Iodophenoxyacetic acid 13734-28-6 19914-26-2
213532-22-0
RL: RCT (Reactant)
(prepn. of radioiodinated Park nucleotide analog as new tool for antibacterial screen development)

IT 16965-06-3P 202464-68-4P 213532-15-1P 213532-16-2P
213532-17-3P 213532-18-4P 213532-19-5P 213532-20-8P
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation)
(prepn. of radioiodinated Park nucleotide analog as new tool for antibacterial screen development)

(FILE 'MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 16:28:04 ON 21 JUL 2000)

L3 4 S L2
L4 2 DUP REM L3 (2 DUPLICATES REMOVED)

L4 ANSWER 1 OF 2 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-096905 [08] WPIDS
DOC. NO. CPI: C2000-028088
TITLE: Novel assay used for diagnosing disease caused by betalactam resistant bacteria.
DERWENT CLASS: B04 D16
INVENTOR(S): DESOUSA, S; PRAHLAD, D
PATENT ASSIGNEE(S): (ASTR) ASTRA AB
COUNTRY COUNT: 86
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9960155	A1	19991125	(200008)*	EN	20

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW

AU 9944029	A	19991206	(200019)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9960155	A1	WO 1999-SE749	19990504
AU 9944029	A	AU 1999-44029	19990504

FILING DETAILS:

Searcher : Shears 308-4994

PATENT NO	KIND		PATENT NO
AU 9944029	A	Based on	WO 9960155

PRIORITY APPLN. INFO: SE 1998-2210 19980622; IN 1998-1019 19980515

AN 2000-096905 [08] WPIDS

AB WO 9960155 A UPAB: 20000215

NOVELTY - A **scintillation** proximity **assay** (**SPA**) used to detect **peptidoglycan** synthesis, is new.

USE - SPA is used for screening beta -lactams, to measure concentration of beta -lactam antibiotics and to measure the activity of beta -lactamases. The assay is also used for diagnosing disease causing beta -lactam resistant bacteria and for identifying inhibitors of beta -lactamases.

ADVANTAGE - The assay is fast, simple, reproducible and highly suitable for screening compounds affecting **peptidoglycan** synthesis.

Dwg.0/8

L4 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 1

ACCESSION NUMBER: 1998:401917 BIOSIS

DOCUMENT NUMBER: PREV199800401917

TITLE: Synthesis of a radioiodinated park nucleotide analog: A new tool for antibacterial screen development.

AUTHOR(S): Eid, Clark N. (1); Nesler, Michael J.; Zia-Ebrahimi, Mohammad; Wu, Chuyn-Yeh Ernie; Yao, Raymond; Cox, Karen; Richardson, John

CORPORATE SOURCE: (1) Bristol-Myers Squibb, 5 Research Parkway, Wallingford, CT 06492-7660 USA

SOURCE: Journal of Labelled Compounds and Radiopharmaceuticals, (Aug., 1998) Vol. 41, No. 8, pp. 705-716.
ISSN: 0362-4803.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The Park nucleotide is an important biological building block used in the construction of bacterial cell walls. Herein, we describe the synthesis of a radiolabeled Park nucleotide analog, p-iodophenoxyacyl-Ala-(D)-iso-Glu-Lys-(D)-Ala-(D)-A:a-OH-(125I), using electrophilic destannylation. Anti-Park nucleotide antibody binding assays using a **scintillation** proximity **assay** (**SPA**) system showed good recognition of the radiolabeled surrogate. This methodology could be used for establishing a screen to identify inhibitors of **peptidoglycan** biosynthesis.

```
     FILE 'REGISTRY' ENTERED AT 16:36:14 ON 21 JUL 2000
                E PEPTIDOGLYCANS/CN
L13             1 S E3

     FILE 'CAPLUS' ENTERED AT 16:36:47 ON 21 JUL 2000
L14             0 S L1 AND L13

     FILE 'MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH,
     JICST-EPLUS, JAPIO' ENTERED AT 16:37:32 ON 21 JUL 2000
L15             0 S L14

     FILE 'REGISTRY' ENTERED AT 16:29:06 ON 21 JUL 2000                    claims 2&3
                E "UDP-N-ACETYLMURAMYLPENTAPEPTIDE"/CN
                E "UDP-N-ACETYLMURAMYL-PENTAPEPTIDE"/CN
L5              0 S ?"DIMINOPIMELIC ACID-D-ALANINE-D-ALANINE"?/CNS
L6              0 S ?"DIAMINOPIMELIC ACID-D-ALANINE-D-ALANINE"?/CNS
                E "URIDINE-5-DIPHOSPHATE-N-ACETYLMURAMYLPENTAPEPTIDE"/CN

                E "UDP-N-ACETYL GLUCOSAMINE"/CN
                E "UDP-N-ACETYLGLUCOSAMINE"/CN
L17             1 S E3

     FILE 'CAPLUS' ENTERED AT 16:40:23 ON 21 JUL 2000
L7          12360 SEA FILE=CAPLUS ABB=ON  PLU=ON  UDP OR URIDINE(W)5(W)(DIP
                  HOSPHATE OR DI PHOSPHATE)
L9             23 SEA FILE=CAPLUS ABB=ON  PLU=ON  L7(S)(DIAMINOPIMELIC OR  <- claim 3
                  DI(W)AMINOPIMELIC)
L11            33 SEA FILE=CAPLUS ABB=ON  PLU=ON  L7(S)(ACETYLMURAMYLPENTA? <- claim 2
                   OR ACETYL(W)(MURAMYLPENTA? OR MURAMYL PENTA?))
L13             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  PEPTIDOGLYCANS/CN
L16            43 SEA FILE=CAPLUS ABB=ON  PLU=ON  (L9 OR L11) AND (L13 OR
                  PEPTIDOGLYCAN OR PEPTIDO GLYCAN)
L17             1 SEA FILE=REGISTRY ABB=ON  PLU=ON  UDP-N-ACETYLGLUCOSAMINE
                  /CN
L18            17 SEA FILE=CAPLUS ABB=ON  PLU=ON  L16 AND (L17 OR (UDP OR
                  URIDINE)(3W)(ACETYLGLUCOSAMINE OR ACETYL GLUCOSAMINE))

=> s l18 not l2

L19           17 L18 NOT L2

=> d 1-17 .bevstr

L19  ANSWER 1 OF 17  CAPLUS  COPYRIGHT 2000 ACS
ACCESSION NUMBER:         1998:131550  CAPLUS
DOCUMENT NUMBER:          128:150907
TITLE:                    Substrate synthesis and activity assay for MurG
AUTHOR(S):                Men, Hongbin; Park, Peter; Ge, Min; Walker,
                          Suzanne
                             Searcher   :    Shears   308-4994
```

CORPORATE SOURCE: Department of Chemistry, Princeton University, Princeton, NJ, 08544, USA
SOURCE: J. Am. Chem. Soc. (1998), 120(10), 2484-2485
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Some of the best antibiotics function by interfering with the biosynthesis of the **peptidoglycan** polymer that surrounds bacterial cells. With the emergence of bacterial pathogens that are resistant to common antibiotics it has become imperative to learn more about the enzymes involved in **peptidoglycan** biosynthesis. Unfortunately, many of the enzymes have proven exceedingly difficult to study. One such enzyme is MurG, a cytoplasmic membrane-assocd. enzyme that transfers **UDP**-N-**acetylglucosamine** to the C4 hydroxyl of a lipid-linked muramic acid deriv. MurG may be termed **UDP**-N-**acetylglucosamine**:N-acetylmuramyl(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase. Because the lipid-linked substrate for MurG is extremely difficult to isolate, no direct assays for MurG activity have been developed. Consequently, it has not been possible to purify MurG or to det. the minimal functional length; nor has it been possible to carry out any detailed mechanistic studies, or to det. the substrate requirements. Here we report the synthesis of a substrate for MurG and show that it can be used in a direct and rapid assay for enzyme activity. This substrate and activity assay should make possible detailed mechanistic and structural analyses of the wholly or partially purified MurG enzyme.

L19 ANSWER 2 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1994:430988 CAPLUS
DOCUMENT NUMBER: 121:30988
TITLE: Analysis of **peptidoglycan** precursors in vancomycin-resistant Enterococcus gallinarum BM4174
AUTHOR(S): Reynolds, Peter E.; Snaith, Hilary A.; Maguire, Alison J.; Dutka-Malen, Sylvie; Courvalin, Patrice
CORPORATE SOURCE: Dep. Biochem., Univ. Cambridge, Cambridge, CB2 1QW, UK
SOURCE: Biochem. J. (1994), 301(1), 5-8
CODEN: BIJOAK; ISSN: 0264-6021
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Vancomycin resistance in enterococci is an increasing clin. problem, and several phenotypes have been identified. The authors demonstrate here that the resistance mechanism in the constitutively vancomycin-resistant Enterococcus gallinarum BM4174 involves an

altered pathway of **peptidoglycan** synthesis and hydrolysis of the normal precursors in the vancomycin-sensitive pathway. A ligase encoded by the vanC gene catalyzes synthesis of D-Ala-D-Ser and substitutes this dipeptide for D-Ala-D-Ala in **peptidoglycan** precursors. It is presumed that this substitution lowers the affinity of vancomycin for its target site. Destruction of D-Ala-D-Ala (D,D-peptidase activity) and of UDP-MurNAc-L-Ala-D-isoGlu-L-Lys-D-Ala-D-Ala by removal of the terminal D-Ala residue (D,D-carboxypeptidase activity) ensures that the normal vancomycin-sensitive pathway of **peptidoglycan** synthesis cannot function in the resistant strain.

IT **528-04-1, UDP-N-acetylglucosamine**
RL: BIOL (Biological study)
(**peptidoglycan** precursor, of vancomycin-resistant Enterococcus gallinarum)

L19 ANSWER 3 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1990:587891 CAPLUS
DOCUMENT NUMBER: 113:187891
TITLE: Inhibition of **peptidoglycan** biosynthesis in Bacillus megaterium by daptomycin
AUTHOR(S): Mengin-Lecreulx, Dominique; Allen, N. E.; Hobbs, J. N.; Van Heijenoort, Jean
CORPORATE SOURCE: Univ. Paris-Sud, Orsay, 91405, Fr.
SOURCE: FEMS Microbiol. Lett. (1990), 69(3), 245-8
CODEN: FMLED7; ISSN: 0378-1097
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The effects of daptomycin on exponential phase cells of B. megaterium were investigated. Bacteriostasis was obsd. for concns. between 1 and 3 .mu.g/mL and maximal rate of cell lysis at 10 .mu.g/mL. At sublytic concns. (1.5-3 .mu.g/mL), the variations of the pools of **UDP**-N-acetylgucosamine and **UDP**-N-**acetylmuramylpentapeptide**, as well as the incorporation of (14C)-N-acetylglucosamine into **peptidoglycan** were studied. It was concluded that the lethal target of daptomycin could be a metabolic step between glucosamine 6-phosphate and **UDP**-N-**acetylglucosamine**.

IT **528-04-1, UDP-N-acetylglucosamine**
RL: BIOL (Biological study)
(**peptidoglycan** formation from, by Bacillus megaterium, daptomycin effect on)

L19 ANSWER 4 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1986:403306 CAPLUS
DOCUMENT NUMBER: 105:3306
TITLE: **Peptidoglycan** synthetic activities in membranes of Escherichia coli caused by

overproduction of penicillin-binding protein 2 and RodA protein

AUTHOR(S): Ishino, Fumitoshi; Park, Wan; Tomioka, Shigeo; Tamaki, Shigeo; Takase, Ichiro; Kunugita, Kiyohiko; Matsuzawa, Hiroshi; Asoh, Sadamitsu; Ohta, Takahisa; et al.

CORPORATE SOURCE: Inst. Appl. Microbiol., Univ. Tokyo, Tokyo, 113, Japan

SOURCE: J. Biol. Chem. (1986), 261(15), 7024-31
CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Penicillin-binding protein 2 (PBP-2) and the RodA protein are known to function in detg. the rod shape of E. coli cells. **Peptidoglycan** biosynthetic reactions that required these 2 proteins were demonstrated in the membrane fraction prepd. from an E. coli strain that overproduced both of these 2 proteins and which lacked PBP-1B activity (the major **peptidoglycan** synthetase activity in the normal E. coli membranes). The cross-linked **peptidoglycan** was synthesized from **UDP-N-acetylmuramylpentapeptide** and **UDP-N-acetylglucosamine** in the presence of a high concn. of cefmetazole that inhibited all the PBPs except PBP-2. The **peptidoglycan** was synthesized via a lipid intermediate and showed .ltoreq.30% cross-linking. The cross-linking reaction was strongly inhibited by amidinopenicillin, mecillinam, and other .beta.-lactam antibiotics that have a high affinity for PBP-2 but not by .beta.-lactams that had very low affinity for PBP-2. The formation of **peptidoglycan** required the presence of high levels of both PBP-2 and the RodA protein in the membranes, but it is unclear which of the 2 proteins was primarily responsible for the extension of the glycan chains (transglycosylation). However, the sensitivity of the cross-linking reaction to specific .beta.-lactam antibiotics strongly suggested that it was catalyzed by PBP-2. The transglycosylase activity of the membranes was sensitive to enramycin and vancomycin and was unusual in being stimulated greatly by a high concn. of a chelating agent.

L19 ANSWER 5 OF 17 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1986:65555 CAPLUS

DOCUMENT NUMBER: 104:65555

TITLE: Isolation of differentiated membrane domains from Escherichia coli and Salmonella typhimurium, including a fraction containing attachment sites between the inner and outer membranes and the murein skeleton of the cell envelope

AUTHOR(S): Ishidate, Kohei; Creeger, Elva S.; Zrike, Joanne; Deb, Sumitra; Glauner, Bernd;

MacAlister, Thomas J.; Rothfield, Lawrence I.
CORPORATE SOURCE: Dep. Microbiol., Univ. Connecticut Health Cent., Farmington, CT, 06032, USA
SOURCE: J. Biol. Chem. (1986), 261(1), 428-43
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Cell envelopes of S. typhimurium and E. coli were disrupted in a French pressure cell and fractionated by successive cycles of sedimentation and flotation d. gradient centrifugation. This method permitted the identification and isolation of several membrane fractions in addn. to the major inner membrane and murein-outer membrane fractions. One of these fractions (fraction OML) accounted for .apprx.10% of the total cell envelope protein and is likely to include the murein-membrane adhesion zones that are seen in electron micrographs of plasmolyzed cells. Fraction OML contained inner membrane, murein, and outer membrane in an apparently normal configuration, was capable of synthesizing murine from **UDP** -3H-labeled N-**acetylglucosamine** and **UDP**-N-**acetylmuramylpentapeptide** and covalently linking it to the endogenous murein of the prepn., and showed a labeling pattern in [3H]galactose pulse-chase expts. that was consistent with its action as an intermediate in the movement of newly synthesized lipopolysaccharide from inner membrane to outer membrane. The fractionation procedure also identified 2 new minor membrane fractions, with characteristic protein patterns, that are usually included in the region of the major inner membrane peak in other fractionation procedures but can be sepd. from the major inner membrane fraction and from contaminating flagellar fragments by the subsequent flotation centrifugation steps.

L19 ANSWER 6 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1985:557031 CAPLUS
DOCUMENT NUMBER: 103:157031
TITLE: Effect of growth conditions on **peptidoglycan** content and cytoplasmic steps of its biosynthesis in Escherichia coli
AUTHOR(S): Mengin-Lecreulx, Dominique; Van Heijenoort, Jean
CORPORATE SOURCE: Cent. Natl. Rech. Sci., Univ. Paris Sud, Orsay, 91405, Fr.
SOURCE: J. Bacteriol. (1985), 163(1), 208-12
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To study the control of **peptidoglycan** synthesis in E. coli, metabolic parameters, such as cell **peptidoglycan** content, the pool levels of 7 uridine nucleotide precursors of **peptidoglycan**, and the specific activities of 5 enzymes involved in **peptidoglycan** formation were investigated

under different growth conditions. When exponential-phase cells with generation times of 25-190 min were examd., the **peptidoglycan** content apparently varied as the cell surface area changed, and no significant variation in the pool levels of the nucleotide precursors or in the specific activities of the 5 enzymes were obsd. The **peptidoglycan** of exponential-phase cells accounted for 0.7-0.8% of the dry cell wt., whereas that of stationary-phase cells accounted for 1.4-1.9%. With different growth conditions, the no. of **peptidoglycan** disaccharide peptide units per cell varied from 2.4 .times. 106 to 5.6 .times. 106. The levels of the nucleotide precursor pools and the specific activities of the D-glutamic acid- and D-alanyl-D-alanine-adding enzymes varied little with different growth phases. The specific activities of **UDP-N-acetylglucosamine** transferase, **UDP-N-acetylglucosamine** -enolpyruvate reductase, and the **diaminopimelic** acid-adding enzymes decreased by 20-50% at most in the late stationary phase. The possible importance of the maintenance of a high capacity for **peptidoglycan** synthesis for cell survival under various growth conditions, and a balance between the synthesis and breakdown of **peptidoglycan** during active growth are discussed.

IT **528-04-1**
RL: BIOL (Biological study)
(in **peptidoglycan** formation, during Escherichia coli growth phases)

L19 ANSWER 7 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1984:153601 CAPLUS
DOCUMENT NUMBER: 100:153601
TITLE: Intracellular accumulation of trehalose during streptomycin formation by Streptomyces griseus
AUTHOR(S): Nimi, Osamu; Amano, Yoshiyuki; Ikeda, Atsushi; Yoshimura, Yoshinori; Sugiyama, Masanori; Nomi, Ryosaku
CORPORATE SOURCE: Fac. Eng., Hiroshima Univ., Higashihiroshima, 724, Japan
SOURCE: Agric. Biol. Chem. (1984), 48(2), 285-90
CODEN: ABCHA6; ISSN: 0002-1369
DOCUMENT TYPE: Journal
LANGUAGE: English

AB When washed mycelium of S. griseus HUT 6037 was incubated in 0.5% NaCl soln. contg. D-[1-14C]glucosamine with shaking at 28.degree., the activity of the mycelium to incorporate radioactivity into the cell wall decreased rapidly, whereas that into streptomycin increased. During this physiol. change, **UDP-N-acetylmuramylpentapeptide**, **UDP-N-acetylglucosamine**, glutamic acid, and trehalose accumulated in the mycelium. The latter 2 substances accumulated much more than

the former 2. When the time courses of the activities of incorporation of [14C]glucosamine into the 4 substances, mucopeptide and streptomycin were examd., a decrease in the activity into **peptidoglycan** led to an increase into streptomycin, glutamic acid, and trehalose. In a pH-stat batch culture with a defined medium, trehalose was accumulated in the cell before glucose was consumed. However, after glucose was consumed, the consumption of trehalose began. Streptomycin prodn. continued until intracellular trehalose completely disappeared in spite of the lack of glucose in the culture medium.

IT **528-04-1**
RL: BIOL (Biological study)
(accumulation of, by Streptomyces griseus, streptomycin formation in relation to)

L19 ANSWER 8 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1984:48366 CAPLUS
DOCUMENT NUMBER: 100:48366
TITLE: The complete sequence of murein synthesis in ether treated Escherichia coli
AUTHOR(S): Metz, Renate; Henning, Susanne; Hammes, Walter P.
CORPORATE SOURCE: Inst. Lebensmitteltechnol., Univ. Hohenheim, Stuttgart, D-7000/70, Fed. Rep. Ger.
SOURCE: Arch. Microbiol. (1983), 136(4), 297-9
CODEN: AMICCW; ISSN: 0302-8933
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The in vitro synthesis of murein from the precursors **UDP-N-acetylglucosamine**, L-alanine, D-glutamic acid, and meso-**diaminopimelic** acid was performed with the aid of ether-treated E. coli. This synthesis was sensitive to representative inhibitors of early reactions in the cytoplasm as well as of late reactions in the membrane or the cell wall. The sensitivity was higher than in in vitro systems starting with **UDP**-N-acetylmuramic acid or **UDP**-N-**acetylmuramylpentapeptide**.

IT **528-04-1**
RL: BIOL (Biological study)
(murein formation from, by in vitro Escherichia coli system)

L19 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1979:451257 CAPLUS
DOCUMENT NUMBER: 91:51257
TITLE: Dissociation and reconstitution of membranes synthesizing the **peptidoglycan** of Bacillus megaterium. A protein factor for the polymerization step
AUTHOR(S): Taku, Akio; Fan, David P.

CORPORATE SOURCE: Dep. Genet. Cell Biol., Univ. Minnesota, St. Paul, MN, 55108, USA
SOURCE: J. Biol. Chem. (1979), 254(10), 3991-9
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cholate-solubilized B. megaterium membranes can be reconstituted by dialysis in the presence of Mg2+ to regain .apprx.12% of the original **peptidoglycan** synthetic activity. Bio-Gel A-5m filtration of the solubilized components showed that all of the compds. necessary for **peptidoglycan** synthesis can be dissocd. into material with a mol. wt. of less than .apprx.68,000. With this reconstitution system, an assay was developed for a new protein factor, PG-II, of B. megaterium. This factor could be combined with phospho-N-**acetylmuramylpentapeptide** translocase and N-acetylglucosaminyl transferase to synthesize polymd. **peptidoglycan** from the precursors **UDP**-N-**acetylmuramylpentapeptide** and **UDP**-N-**acetylglucosamine**. In the absence of PG-II, the disaccharide pentapeptide substrate for the polymerase was accumulated. In the presence of this factor, the amt. of the substrate was diminished and polymeric **peptidoglycan** was formed. Therefore, PG-II was likely to be necessary for the polymn. step and may well have been the polymerase itself. From 3 chromatog. steps developed for the purifn. of PG-II, it seemed likely that a single protein with a mol. wt. of .apprx.60,000 could have PG-II activity.

L19 ANSWER 10 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1979:198204 CAPLUS
DOCUMENT NUMBER: 90:198204
TITLE: Amphomycin inhibits phospho-N-acetylmuramyl-pentapeptide translocase in **peptidoglycan** synthesis of Bacillus
AUTHOR(S): Tanaka, Haruo; Oiwa, Ruiko; Matsukura, Shigekazu; Omura, Satoshi
CORPORATE SOURCE: Kitasato Univ., Tokyo, Japan
SOURCE: Biochem. Biophys. Res. Commun. (1979), 86(3), 902-8
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Amphomycin Na salt [63653-28-1], a selective inhibitor of **peptidoglycan** synthesis of bacteria, inhibited the lipid intermediates accumulation and the **peptidoglycan** synthesis from **UDP**-N-acetylmuramyl-L-Ala-D-Glu-meso-**diaminopimelic** acid-D-Ala-D-Ala (**UDP**-MurNAc-pentapeptide) [52683-72-4] and **UDP**-N-**acetylglucosamine** (**UDP**-GlcNAc) [**528-04-1**

] with a particulate fraction from B. megaterium KM, and also inhibited the formation of MurNAc-(pentapeptide)-P-P-lipid in the absence of **UDP**-GlcNAc. But it did not inhibit the formation of **peptidoglycan** from MurnAc-(pentapeptide)-P-P-lipid and UDP-GlcNAc with the same system of the organism. Apparently the site of action of amphomycin is phospho-MurNAc-pentapeptide translocase [70132-27-3] in **peptidoglycan** synthesis.

IT **528-04-1**
RL: PRP (Properties)
(**peptidoglycan** formation from, in Bacillus megaterium, amphomycin inhibition of)

L19 ANSWER 11 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1977:496696 CAPLUS
DOCUMENT NUMBER: 87:96696
TITLE: Studies on bacterial cell wall inhibitors. II. Inhibition of **peptidoglycan** synthesis in vivo and in vitro by amphomycin
AUTHOR(S): Tanaka, Haruo; Iwai, Yuzuru; Oiwa, Ruiko; Shinohara, Shoji; Shimizu, Shoji; Oka, Tetsuo; Omura, Satoshi
CORPORATE SOURCE: Kitasato Univ., Tokyo, Japan
SOURCE: Biochim. Biophys. Acta (1977), 497(3), 633-40
CODEN: BBACAQ
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Sodium amphomycin [63653-28-1] (10 .mu.g/mL) lysed growing cells of Bacillus cereus T and inhibited **peptidoglycan** synthesis, accompanied by accumulation of uridine diphosphate-N-acetylmuramyl (UDP-MurNAc) peptides. The nucleotide precursors that accumulated in cells of Staphylococcus aureus in the presence of amphomycin were identified as UDP-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala, UDP-MurNAc-L-Ala, and UDP-MurNAc. In expts. using a particulate enzyme system of B. megaterium, amphomycin inhibited the polymn. of **UDP**-MurNAc-L-Ala-D-Glu-meso-**diaminopimelic** acid-D-Ala-D-Ala (**UDP**-MurNAc-pentapeptide) and **UDP**-N-**acetylglucosamine**, and also inhibited the formation of lipid intermediates, but did not inhibit the cross-linking, the last step of **peptidoglycan** synthesis. Unlike bacitracin [1405-87-4], amphomycin did not lyse protoplats of B. megaterium. The site of action of amphomycin is apparently the formation of MurNAc-(pentapeptide)-P-P-lipid from MurNAc-pentapeptide and undecaprenol (lipid) phosphate.

L19 ANSWER 12 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1977:26406 CAPLUS
DOCUMENT NUMBER: 86:26406
TITLE: Biosynthesis of **peptidoglycan** in

Gaffkya homari. The mode of action of penicillin G and mecillinam

AUTHOR(S): Hammes, Walter P.

CORPORATE SOURCE: Bot. Inst., Univ. Muenchen, Munich, Ger.

SOURCE: Eur. J. Biochem. (1976), 70(1), 107-13
CODEN: EJBCAI

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The effect of the .beta.-lactam antibiotics penicillin G [61-33-6] and mecillinam [32887-01-7] on the incorporation of **peptidoglycan** into pre-formed cell wall **peptidoglycan** was studied with wall membrane enzyme prepns. from G. homari. With **UDP**-N-**acetylglucosamine** (**UDP**-GlcNAc) and **UDP**-N-**acetylmuramylpentapeptide** (**UDP**-MurNAc-pentapeptide) as precursors, the incorporation of **peptidoglycan** into the pre-existing cell wall of G. homari was inhibited to an extent of 50% (ID50 value) at a concn. of 0.25 .mu.g of penicillin G/ml. With UDP-GlcNAc and UDP-MurNAc-tetrapeptide as precursors, the ID50 value was about 2500-fold greater (630 .mu.g/m). The inhibition by penicillin G of the incorporation of **peptidoglycan** from UDP-MurNAc-[14C]Lys-pentapeptide could be overcome by addn. of non-radioactive UDP-MurNAc-tetrapeptide to the incubation mixt. In the presence of 5 .mu.g of penicillin G/ml the incorporation of **peptidoglycan** formed from the mixt. of UDP-MurNAc-Ala-D-Glu-Lys-D-[14C]Ala-D-[14C]Ala and nonradioactive UDP-MurNAc-tetrapeptide proceeded virtually without release of D-[14C]alanine by transpeptidase activity. The enzyme prepn. also exhibited DD-carboxypeptidase [9077-67-2] activity which was only slightly more sensitive to penicillin G and mecillinam than was the incorporation of **peptidoglycan** into the cell wall. Since the ID50 values for the .beta.-lactam antibiotics are similar to the concns. required to inhibit the growth of G. homari to an extent of 50%, the DD-carboxypeptidase must be the killing site of both penicillin G and mecillinam.

L19 ANSWER 13 OF 17 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1975:166493 CAPLUS

DOCUMENT NUMBER: 82:166493

TITLE: Biosynthesis of **uridine** diphospho-N-**acetylglucosamine**. V. Pyruvate-**uridine** diphospho-N-**acetylglucosamine** transferase. Purification to homogeneity and feedback inhibition

AUTHOR(S): Zemell, Ronald I.; Anwar, Rashid A.

CORPORATE SOURCE: Dep. Biochem., Univ. Toronto, Toronto, Ont., Can.

SOURCE: J. Biol. Chem. (1975), 250(8), 3185-92
CODEN: JBCHA3
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The title enzyme was purified to homogeneity from Enterobacter cloacae with **UDP**-N-acetylmuramyl-L-Ala-D-Glu-meso-Dap, (Dap = **diaminopimelic** acid), a feedback inhibitor, as a ligand covalently bound to Sepharose 4B. The enzyme was a single polypeptide with a mol. wt. of 41,000. The enzyme catalyzed the 1st committed step in the biosynthesis of bacterial cell wall **peptidoglycan**. The cytoplasmic end product of this pathway was UDP-N-acetylmuramyl-L-Ala-D-Glu-meso-Dap-D-Ala-D-Ala (UDP-MurNAc-pentapeptide). UDP-MurNAc-pentapeptide and its precursor, UDP-MurNAc-tripeptide, were effective inhibitors of the enzyme. The kinetic data suggested a binding site for these inhibitors distinct from the active site. This is consistent with the proposed role for UDP-MurNAc-tripeptide and pentapeptide as neg. modulators of the enzyme.

L19 ANSWER 14 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1974:79945 CAPLUS
DOCUMENT NUMBER: 80:79945
TITLE: Control of synthesis of bacterial cell walls. Interaction in the synthesis of nucleotide precursors
AUTHOR(S): Anderson, Raymond G.; Douglas, L. Julia; Hussey, Helen; Baddiley, James
CORPORATE SOURCE: Microbiol. Chem. Res. Lab., Univ. Newcastle-upon-Tyne, Newcastle-upon-Tyne, Engl.
SOURCE: Biochem. J. (1973), 136(4), 871-6
CODEN: BIJOAK
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Enzymes involved in forming **peptidoglycan** and teichoic acid are regulated by precursors of the alternative product. In sol. exts of Bacillus licheniformis ATCC 9945 phosphoenolpyruvate **UDP**-N-**acetylglucosamine** enolpyruvyltransferase was inhibited by **UDP-acetylmuramylpentapeptide**, **UDP**-N-**acetylglucosamine** pyrophosphorylase was inhibited by the pentapeptide and, in a concn.-dependent manner, by CDP-glycerol, and CDP-glycerol pyrophosphorylase was inhibited by the pentapeptide and CDP-glycerol and stimulated by **UDP**-N-**acetylglucosamine**.

L19 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1972:537187 CAPLUS
DOCUMENT NUMBER: 77:137187
TITLE: Biosynthesis of the **peptidoglycan** of bacterial cell walls. XXX.

Penicillin-sensitive transpeptidation during **peptidoglycan** biosynthesis in cell-free preparations from Bacillus megaterium. I. Incorporation of free diaminopimelic acid into **peptidoglycan**

AUTHOR(S): Wickus, Gary G.; Strominger, Jack L.
CORPORATE SOURCE: Biol. Lab., Harvard Univ., Cambridge, Mass., USA
SOURCE: J. Biol. Chem. (1972), 247(17), 5297-306
CODEN: JBCHA3
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A particulate enzyme system for B. megaterium QMB1551 was described which catalyzes the utiliza-tion of the uridine nucleotides, **UDP**-N-acetylmuramyl-L-Ala-D-Glu-meso-**diaminopimelic** acid-D-Ala-D-Ala and **UDP**-N-**acetyl-glucosamine**, for **peptidoglycan** synthesis. Unlike systems previously studied in gram-pos. microorganisms, this particulate enzyme prepn. catalyzed the terminal cross-linking reaction in cell wall biosynthesis. This system could also incorporate free diaminopimelic acid dependent on the formation of **peptidoglycan** polymer from the uridine nucleotide substrates but independent of ATP. Furthermore, the incorporation of diaminopimelic acid was inhibited by penicillins and the diaminopimelic acid appeared to be incorporated onto a terminal position of the peptide of a di-saccharide-pentapeptide **peptidoglycan** unit with the release of alanine. The disaccharide-peptide products formed during the incorporation of free diaminopimelic acid were isolated from **peptidoglycan** and analyzed. A reaction sequence for incorpora-tion of free diaminopimelic acid into **peptidoglycan** is proposed.

L19 ANSWER 16 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1972:523203 CAPLUS
DOCUMENT NUMBER: 77:123203
TITLE: Biosynthesis of the **peptidoglycan** of bacterial cell walls. XXII. Activation of D-aspartic acid for incorporation into **peptidoglycan**

AUTHOR(S): Staudenbauer, Walter; Strominger, Jack L.
CORPORATE SOURCE: Biol. Lab., Harvard Univ., Cambridge, Mass., USA
SOURCE: J. Biol. Chem. (1972), 247(16), 5095-102
CODEN: JBCHA3
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enzyme prepns. from Streptococcus faecalis and Lactobacillus casei catalyzed the incorporation of D-aspartic acid into a **peptidoglycan** in the presence of **UDP-acetylmuramylpentapeptide** and **UDP-acetylglucosamine**. The reaction required ATP but was

independent of either supernatant enzymes or tRNA. A D-aspartic acid-activating enzyme present in the membrane fraction was solubilized with 2M LiCl and purified approx. 100-fold. The prepn. so obtained catalyzed the formation of equiv. amts. of .beta.-D-aspartylhydroxamate, ADP, and inorg. phosphate in the presence of D-aspartic acid, ATP, and hydroxylamine. The intermediate formed is presumably .beta.-D-aspartylphosphate.

L19 ANSWER 17 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1967:440234 CAPLUS
DOCUMENT NUMBER: 67:40234
TITLE: Biosynthesis of the **peptidoglycan** of bacterial cell walls. IV. Incorporation of glycine in Micrococcus lysodeikticus
AUTHOR(S): Katz, Walther; Matsuhashi, Michio; Dietrich, Carl P.; Strominger, Jack L.
CORPORATE SOURCE: Univ. of Wisconsin Med. Sch., Madison, Wis., USA
SOURCE: J. Biol. Chem. (1967), 242(13), 3207-17
CODEN: JBCHA3
DOCUMENT TYPE: Journal
LANGUAGE: English
AB cf. preceding abstr. A system has been described for the incorporation of glycine into the **peptidoglycan** catalyzed by particulate enzymes from M. lysodeikticus. Glycine incorporation required both of the uridine nucleotides required for **peptidoglycan** synthesis, uridine di-phosphate **acetylmuramylpentapeptide** (**UDP**-MurNAc-pentapeptide) and **uridine** diphosphate **acetylglucosamine** (**UDP**-Glc-NAc), as well as ATP. It was independent of addn. of supernatant soln. or sol. RNA. The glycine residue was incorporated into the product in a COOH-terminal position. Since it was not attached to the terminal D-alanine residue of Mur-NAc-pentapeptide, it must have been attached to the only other available carboxyl group, the .alpha.-carboxyl group of glutamic acid. This point of attachment is in agreement with structural studies of the **peptidoglycan** of this organism. It has been demonstrated that the immediate acceptor of glycine is the lipid, disaccharide(-pentapeptide)-P-P-phospholipid, previously shown to be an intermediate in **peptidoglycan** synthesis. Some properties of the reaction have been investigated with the isolated lipid intermediate as acceptor in the absence of added uridine nucleotides. Although MurNAc(-pentapeptide)-P-P-phospholipid also accepted glycine, it was not as effective an acceptor as the disaccharide-contg. lipid. The glycine-14C-labeled product of the reaction, disaccharide-hexapeptide-14C-P-P-phospholipid, was also isolated and shown to be utilized for **peptidoglycan** synthesis in the absence of glycine, ATP, or added uridine nucleotides. The arsenolysis of this product in the presence of ADP suggests that ADP and inorg. phosphate are the

products of the forward reaction, although their formation could not be measured because of simultaneous ATPase activity. 21 references.

IT **528-04-1**
RL: BIOL (Biological study)
(in glycopeptide formation from glycine by Micrococcus lysodeikticus)

(FILE 'MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 16:44:46 ON 21 JUL 2000)

L20 22 S L18
L21 21 S L20 NOT L3
L22 13 DUP REM L21 (8 DUPLICATES REMOVED)

L22 ANSWER 1 OF 13 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1991:410537 BIOSIS
DOCUMENT NUMBER: BA92:77502
TITLE: THE MURG GENE OF ESCHERICHIA-COLI CODES FOR THE **UDP-N-ACETYLGLUCOSAMINE** N **ACETYLMURAMYLPENTAPEPTIDE** PYROPHOSPHORYL-UNDECAPRENOL N-ACETYLGLUCOSAMINE TRANSFERASE INVOLVED IN THE MEMBRANE STEPS OF **PEPTIDOGLYCAN** SYNTHESIS.
AUTHOR(S): MENGIN-LECREULX D L T; ROUSSEAU M; VAN HEIJENOORT J
CORPORATE SOURCE: LABORATOIRE BIOCHIMIE MOLECULAIRE CELLULAIRE, URA 1131, CENTRE NATIONAL RECHERCHE SCIENTIFIQUE, UNIVERSITE PARIS-SUD, BATIMENT 432, ORSAY, FR.
SOURCE: J BACTERIOL, (1991) 173 (15), 4625-4636.
CODEN: JOBAAY. ISSN: 0021-9193.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Physiological properties of the murG gene product of Escherichia coli were investigated. The inactivation of the murG gene rapidly inhibits **peptidoglycan** synthesis in exponentially growing cells. As a result, various alterations of cell shape are observed, and cell lysis finally occurs when the **peptidoglycan** content is 40% lower than that of normally growing cells. Analysis of the pools of **peptidoglycan** precursors reveals the concomitant accumulation of **UDP-N-acetylglucosamine** (UDP-GlcNAc) and UDP-N-acetylmuramyl-pentapeptide (UDP-MurNAc-pentapeptide) and, to a lesser extent, that of undecaprenyl-pyrophosphoryl-MurNAc-pentapeptide (lipid intermediate I), indicating that inhibition of **peptidoglycan** synthesis occurs after formation of the cytoplasmic precursors. The relative depletion of the second lipid intermediate, undecaprenyl-pyrophosphoryl-MurNAc-(pentapeptide)GlcNAc, shows that inactivation of the murG gene product does not prevent the formation of lipid intermediate I but inhibits the next reaction in which GlcNAc is transferred to lipid intermediate I. In vitro assays for phospho-MurNAc-pentapeptide translocase and N-acetylglucosaminyl

transferase activities finally confirm the identification of the murG gene product as the transferase that catalyzes the conversion of lipid intermediate I to lipid intermediate II in the **peptidoglycan** synthesis pathway. Plasmids allowing for a high overproduction of the transferase and the determination of its N-terminal amino acid sequence were constructed. In cell fractionation experiments, the transferase is essentially associated with membranes when it is recovered.

L22 ANSWER 2 OF 13 MEDLINE
ACCESSION NUMBER: 91258324 MEDLINE
DOCUMENT NUMBER: 91258324
TITLE: Analysis of murein and murein precursors during antibiotic-induced lysis of Escherichia coli.
AUTHOR: Kohlrausch U; Holtje J V
CORPORATE SOURCE: Abteilung Biochemie, Max-Planck-Institut fur Entwicklungsbiologie, Federal Republic of Germany..
SOURCE: JOURNAL OF BACTERIOLOGY, (1991 Jun) 173 (11) 3425-31. Journal code: HH3. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199109

AB Lysis of Escherichia coli induced by either D-cycloserine, moenomycin, or penicillin G was monitored by studying murein metabolism. The levels of the soluble murein precursor **UDP**-N-acetylmuramyl-L-alanyl-D-glutamyl-m-diaminopimelyl-D-alanyl-D-alanine (**UDP**-MurNAc-pentapeptide) and the carrier-linked MurNAc-(pentapeptide)-pyrophosphoryl-undecaprenol as well as N-acetylglucosamine-beta-1,4-MurNAc-(pentapeptide)-pyrophosphoryl-undecaprenol varied in a specific way. In the presence of penicillin, which is known to interfere with the cross-linking of murein, the concentration of the lipid-linked precursors unexpectedly decreased before the onset of lysis, although the level of **UDP**-MurNAc-pentapeptide remained normal. In the case of moenomycin, which specifically blocks the formation of the murein polysaccharide strands, the lipid-linked precursors as well as **UDP**-MurNAc-pentapeptide accumulated as was expected. D-Cycloserine, which inhibits the biosynthesis of **UDP**-MurNAc-pentapeptide, consequently caused a decrease in all three precursors. The muropeptide composition of the murein showed general changes such as an increase in the unusual DL-cross bridge between two neighboring meso-**diaminopimelic** acid residues and, as a result of uncontrolled DL- and DD-carboxypeptidase activity, an increase in tripeptidyl and a decrease in tetrapeptidyl and pentapeptidyl moieties. The average length of the glycan strands decreased. When the glycan strands were fractionated according to length, a dramatic increase in the amount of single disaccharide

units was observed not only in the presence of penicillin but also in the presence of moenomycin. This result is explained by the action of an exo-muramidase, such as the lytic transglycosylases present in E. coli. It is proposed that antibiotic-induced bacteriolysis is the result of a zipperlike splitting of the murein net by exo-muramidases locally restricted to the equatorial zone of the cell.

L22 ANSWER 3 OF 13 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 90207850 EMBASE
DOCUMENT NUMBER: 1990207850
TITLE: Inhibition of **peptidoglycan** biosynthesis in Bacillus megaterium by daptomycin.
AUTHOR: Mengin-Lecreulx D.; Allen N.E.; Hobbs J.N.; Van Heijenoort J.
CORPORATE SOURCE: Unite de Rech. Ass. du CNRS, Biochimie Molec. et Cell., Universite Paris-Sud,91405 Orsay, France
SOURCE: FEMS Microbiology Letters, (1990) 69/3 (245-248). ISSN: 0378-1097 CODEN: FMLED7
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The effects of daptomycin on exponential phase cells of Bacillus megaterium were investigated. Bacteriostasis was observed for concentration between 1 and 3 .mu.g/ml and maximal rate of cell lysis at 10 .mu.g/ml. At sublytic concentrations (1.5-3 .mu.g/ml), the variations of the pools of **UDP**-N-**acetylglucosamine** and **UDP**-N-**acetylmuramylpentapetide**, as well as the incorporation of (14C)-N-acetylglucosamine into **peptidoglycan** were studied. From the results it was concluded that the lethal target of daptomycin could be a metabolic step between glucosamine 6-phosphate and **UDP**-N-**acetylglucosamine**.

L22 ANSWER 4 OF 13 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1989:339509 BIOSIS
DOCUMENT NUMBER: BA88:42509
TITLE: VARIATIONS IN **UDP**-N-**ACETYLGLUCOSAMINE** AND **UDP**-N-**ACETYLMURAMYLPENTAPEPTIDE** POOLS IN ESCHERICHIA-COLI AFTER INHIBITION OF PROTEIN SYNTHESIS.
AUTHOR(S): MENGIN-LECREULX D; SIEGEL E; VAN HEIJENOORT J
CORPORATE SOURCE: UNITE ASSOCIEE 1131 DU CENT. NATL. DE LA RECHERCHE SCI., BIOCHIMIE MOL. ET CELLULAIRE, BATIMENT 432, UNIVERSITE PARIS-SUD, 91405 ORSAY, FRANCE.

SOURCE: J BACTERIOL, (1989) 171 (6), 3282-3287.
CODEN: JOBAAY. ISSN: 0021-9193.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB The pool levels of the nucleotide precursors of **peptidoglycan** were analyzed after inhibition of protein synthesis in various Escherichia coli strains. In all cases **UDP-N-acetylglucosamine** (UDP-GlcNAc) and UDP-N-acetylmuramyl-pentapeptide (UDP-MurNAc-pentapeptide) cell pools increased upon treatment with chloramphenicol or tetracycline. Similar results were observed after the treatment of K-12 strains with valine. Since the intermediate nucleotide precursors did not accumulate after the arrest of protein synthesis and since a feedback mechanism was unlikely, the increases of the UDP-MurNAc-pentapeptide pool appeared as a consequence of that of the UDP-GlcNAc pool by the unrestricted functioning of the intermediate steps of the pathway. The highest increase (sixfold) of UDP-GlcNAc was oserved with strain K-12 HfrH growing in minimal medium and treated with chloramphenicol. When a pair of isogenic Rel+ and Rel- strains were considered, both the UDP-GlcNAc and UDP-MurNAc-pentapeptide pools increased upon treatment with chloramphenicol or valine. However, the UDP-GlcNAc pool of the Rel+ strain was at a high natural level, which increased only moderately (20%) after the addition of valine. The increase of the UDP-GlcNAc pool after the various treatments could be due to an effect on some upstream step by an unknown mechanism. The possible correlations of the variations of the precursor pools with the rate of synthesis and extent of cross-linking of **peptidoglycan** were also considered.

L22 ANSWER 5 OF 13 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 86196149 MEDLINE
DOCUMENT NUMBER: 86196149
TITLE: **Peptidoglycan** synthetic activities in membranes of Escherichia coli caused by overproduction of penicillin-binding protein 2 and rodA protein.
AUTHOR: Ishino F; Park W; Tomioka S; Tamaki S; Takase I; Kunugita K; Matsuzawa H; Asoh S; Ohta T; Spratt B G; et al
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1986 May 25) 261 (15) 7024-31.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 198608

AB Penicillin-binding protein (PBP)-2 and the RodA protein are known to

function in determining the rod shape of Escherichia coli cells. **Peptidoglycan** biosynthetic reactions that required these two proteins were demonstrated in the membrane fraction prepared from an E. coli strain that overproduced both of these two proteins and which lacked PBP-1B activity (the major **peptidoglycan** synthetase activity in the normal E. coli membranes). The cross-linked **peptidoglycan** was synthesized from **UDP-N-acetylmuramylpentapeptide** and **UDP -N-acetylglucosamine** in the presence of a high concentration of cefmetazole that inhibited all of PBPs except PBP-2. The **peptidoglycan** was synthesized via a lipid intermediate and showed up to 30% cross-linking. The cross-linking reaction was strongly inhibited by the amidinopenicillin, mecillinam, and by other beta-lactam antibiotics that have a high affinity for PBP-2, but not by beta-lactams that had very low affinity for PBP-2. The formation of **peptidoglycan** required the presence of high levels of both PBP-2 and the RodA protein in the membranes, but it is unclear which of the two proteins was primarily responsible for the extension of the glycan chains (transglycosylation). However, the sensitivity of the cross-linking reaction to specific beta-lactam antibiotics strongly suggested that it was catalyzed by PBP-2. The transglycosylase activity of the membranes was sensitive to enramycin and vancomycin and was unusual in being stimulated greatly by a high concentration of a chelating agent.

L22 ANSWER 6 OF 13 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 86085850 MEDLINE
DOCUMENT NUMBER: 86085850
TITLE: Isolation of differentiated membrane domains from Escherichia coli and Salmonella typhimurium, including a fraction containing attachment sites between the inner and outer membranes and the murein skeleton of the cell envelope.
AUTHOR: Ishidate K; Creeger E S; Zrike J; Deb S; Glauner B; MacAlister T J; Rothfield L I
CONTRACT NUMBER: AM-13407 (NIADDK)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1986 Jan 5) 261 (1) 428-43.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 198604
AB Cell envelopes of Salmonella typhimurium and Escherichia coli were disrupted in a French pressure cell and fractionated by successive cycles of sedimentation and floatation density gradient centrifugation. This permitted the identification and isolation of

several membrane fractions in addition to the major inner membrane and murein-outer membrane fractions. One of these fractions (fraction OML) accounted for about 10% of the total cell envelope protein, and is likely to include the murein-membrane adhesion zones that are seen in electron micrographs of plasmolyzed cells. Fraction OML contained inner membrane, murein, and outer membrane in an apparently normal configuration, was capable of synthesizing murein from **UDP**-[3H]N-**acetylglucosamine** and **UDP** -N-**acetylmuramylpentapeptide** and covalently linking it to the endogenous murein of the preparation, and showed a labeling pattern in [3H]galactose pulse-chase experiments that was consistent with its acting as an intermediate in the movement of newly synthesized lipopolysaccharide from inner membrane to outer membrane. The fractionation procedure also identified two new minor membrane fractions, with characteristic protein patterns, that are usually included in the region of the major inner membrane peak in other fractionation procedures but can be separated from the major inner membrane fraction and from contaminating flagellar fragments by the subsequent floatation centrifugation steps.

L22 ANSWER 7 OF 13 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 85234360 MEDLINE
DOCUMENT NUMBER: 85234360
TITLE: Effect of growth conditions on **peptidoglycan** content and cytoplasmic steps of its biosynthesis in Escherichia coli.
AUTHOR: Mengin-Lecreulx D; van Heijenoort J
SOURCE: JOURNAL OF BACTERIOLOGY, (1985 Jul) 163 (1) 208-12. Journal code: HH3. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198510
AB In an attempt to bring some insight into how **peptidoglycan** synthesis is controlled in Escherichia coli, simple parameters, such as cell **peptidoglycan** content, the pool levels of its seven uridine nucleotide precursors, and the specific activities of five enzymes involved in their formation, were investigated under different growth conditions. When exponential-phase cells with generation times ranging from 25 to 190 min were examined, the **peptidoglycan** content apparently varied as the cell surface area changed, and no important variations in the pool levels of the nucleotide precursors or in the specific activities of the five enzymes considered were observed. The **peptidoglycan** of exponential-phase cells accounted for 0.7 to 0.8% of the dry cell weight, whereas that of stationary-phase cells accounted for 1.4 to 1.9%. Depending on the growth conditions, the number of **peptidoglycan** disaccharide peptide units per cell varied

from 2.4 X 10(6) to 5.6 X 10(6). The levels of the nucleotide precursor pools as well as the specific activities of the D-glutamic acid- and D-alanyl-D-alanine-adding enzymes varied little with the growth phase. The specific activities of **UDP**-N-**acetylglucosamine** transferase, **UDP**-N-**acetylglucosamine**-enolpyruvate reductase, and the **diaminopimelic** acid-adding enzymes decreased by 20 to 50% at most in the late stationary phase. The results are discussed in terms of the possible importance for cell survival of the maintenance of a high capacity for **peptidoglycan** synthesis, whatever its rate under various growth conditions, and of a balance between the synthesis and breakdown of **peptidoglycan** during active growth.

L22 ANSWER 8 OF 13 MEDLINE
ACCESSION NUMBER: 84127197 MEDLINE
DOCUMENT NUMBER: 84127197
TITLE: The complete sequence of murein synthesis in ether treated Escherichia coli.
AUTHOR: Metz R; Henning S; Hammes W P
SOURCE: ARCHIVES OF MICROBIOLOGY, (1983 Dec) 136 (4) 297-9. Journal code: 7YN. ISSN: 0302-8933.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198405
AB The in vitro synthesis of murein from the precursors **UDP**-N-**acetylglucosamine**, L-alanine, D-glutamic acid and meso-**diaminopimelic** acid was performed with the aid of ether treated Escherichia coli. This synthesis was sensitive to representative inhibitors of early reactions in the cytoplasm as well as of late reactions in the membrane or the cell wall. The sensitivity was higher than in in vitro systems starting with **UDP**-N-acetylmuramic acid or **UDP**-N-acetylmuramyl-pentapeptide.

L22 ANSWER 9 OF 13 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1979:211380 BIOSIS
DOCUMENT NUMBER: BA68:13884
TITLE: AMPHOMYCIN INHIBITS PHOSPHO-N-ACETYLMURAMYL PENTA PEPTIDE TRANSLOCASE IN **PEPTIDO GLYCAN** SYNTHESIS OF BACILLUS-MEGATERIUM.
AUTHOR(S): TANAKA H; OIWA R; MATSUKURA S; OMURA S
CORPORATE SOURCE: KITASATO INST., MINATO, TOKYO 108, JPN.
SOURCE: BIOCHEM BIOPHYS RES COMMUN, (1979) 86 (3), 902-908. CODEN: BBRCA9. ISSN: 0006-291X.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Amphomycin, a selective inhibitor of **peptidoglycan** synthesis of bacteria, inhibited the lipid intermediates accumulation and the **peptidoglycan** synthesis from **UDP**-N-acetylmuramyl-L-Ala-D-Glu[glucose]-[3H]-meso-Dpm[**diaminopimelic** acid]-D-Ala-D-Ala (**UDP**-MurNAc-pentapeptide) and **UDP**-N-**acetylglucosamine** (**UDP**-GlcNAc) with a particulate fraction from B. megaterium KM, and also inhibited the formation of MurNAc(-pentapeptide)-P-P-lipid in the absence of **UDP**-GlcNAc. But it did not inhibit the formation of **peptidoglycan** from MurNAc(-pentapeptide)-P-P-lipid and **UDP**-GlcNAc with the same system of the organism. The site of action of amphomycin is apparently phospho-MurNAc-pentapeptide translocase in **peptidoglycan** synthesis.

L22 ANSWER 10 OF 13 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 4
ACCESSION NUMBER: 1977:214747 BIOSIS
DOCUMENT NUMBER: BA64:37111
TITLE: STUDIES ON BACTERIAL CELL WALL INHIBITORS PART 2 INHIBITION OF **PEPTIDO GLYCAN** SYNTHESIS IN-VIVO AND IN-VITRO BY AMPHOMYCIN.
AUTHOR(S): TANAKA H; IWAI Y; OIWA R; SHINOHARA S; SHIMIZU S; OKA T; OMURA S
SOURCE: BIOCHIM BIOPHYS ACTA, (1977) 497 (3), 633-640. CODEN: BBACAQ. ISSN: 0006-3002.
FILE SEGMENT: BA; OLD
LANGUAGE: Unavailable
AB Amphomycin was reported by the present authors to be a selective inhibitor of cell wall **peptidoglycan** synthesis in Bacillus cereus T. Investigations were carried out to clarify the target of amphomycin. Amphomycin (10 .mu.g/ml) lysed growing cells of B. cereus T, and inhibited **peptidoglycan** synthesis, accompanied by accumulation of **UDP**-N-acetylmuramyl (**UDP**-MurNAc) peptides. The nucleotide precursors that accumulated in cells of Staphylococcus aureus FDA 209P in the presence of amphomycin were identified as **UDP**-MurNAc-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala, **UDP**-MurNAc-L-Ala and **UDP**-MurNAc. In the experiments using a particulate enzyme system of Bacillus megaterium KM, amphomycin inhibited the polymerization of **UDP**-MurNAc-L-Ala-D-Glu-meso-**diaminopimelic** acid-D-Ala-D-Ala (**UDP**-MurNAc-pentapeptide) and **UDP**-N-**acetylglucosamine** , and also inhibited the formation of lipid intermediates, but did not inhibit the cross-linking, the last step of **peptidoglycan** synthesis. Unlike bacitracin, amphomycin did not lyse protoplasts of B. megaterium KM. The site of action of amphomycin is apparently the formation of MurNAc-(pentapeptide)-P-P-lipid from MurNAc-pentapeptide and undecaprenol (lipid) phosphate.

Searcher : Shears 308-4994

L22 ANSWER 11 OF 13 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 77181393 MEDLINE
DOCUMENT NUMBER: 77181393
TITLE: Chemical structure of the **peptidoglycan** of Vibrio parahaemolyticus A55 with special reference to the extent of interpeptide cross-linking.
AUTHOR: Kato K; Iwata S; Suginaka H; Namba K; Kotani S
SOURCE: BIKEN JOURNAL, (1976 Dec) 19 (4) 139-50.
Journal code: 9XS. ISSN: 0006-2324.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197708
AB The chemical structure of the cell wall **peptidoglycan** of Vibrio parahaemolyticus A55 was studied. Estimation of cross linkages between peptide subunits in the **peptidoglycan** by dinitrophenylation showed that about 30% of the total 2,6-**diaminopimelic** acid (A2pm) residues were involved in cross linkages. The presence of interpeptide bridges was also demonstrated by isolating bisdisaccharide peptide subunit dimers from Chalaropsis muramidase digests of the cell wall **peptidoglycan** by gel filtration followed by ion-exchange column chromatography, although most of the building blocks obtained were uncross-linked disaccharide peptide monomers. The chain length of a glycan moiety of the **peptidoglycan** obtained by treatment with the L-11 enzyme and gel filtration of the digest was also studied. The chain length varied from 7 to 44, but 30% of the glycan fragments had muramic acid at the reducing end and a chain length of 28 to 44. In conformity with the above structural study it was demonstrated that a particulate enzyme fraction obtained by differential centrifugation of a sonicated preparation of V. parahaemolyticus catalyzed a penicillin-sensitive transpeptidation reaction, using **UDP**-MurNAc-14C-pentapeptide and **UDP**-GlcNAc as substrates.

L22 ANSWER 12 OF 13 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 6
ACCESSION NUMBER: 1977:151855 BIOSIS
DOCUMENT NUMBER: BA63:46719
TITLE: BIOSYNTHESIS OF **PEPTIDO GLYCAN** IN GAFFKYA-HOMARI THE MODE OF ACTION OF PENICILLIN G AND MECILLINAM.
AUTHOR(S): HAMMES W P
SOURCE: EUR J BIOCHEM, (1976) 70 (1), 107-113.
CODEN: EJBCAI. ISSN: 0014-2956.
FILE SEGMENT: BA; OLD
LANGUAGE: Unavailable
AB The effect of the .beta.-lactam antibiotics penicillin G and mecillinam on the incorporation of **peptidoglycan** into

pre-formed cell wall **peptidoglycan** was studied with wall membrane enzyme preparations from G. homari. Using **UDP**-N-**acetylglucosamine** (**UDP**-GlcNAc) and **UDP** -N-**acetylmuramylpentapeptide** (**UDP** -MurNAc-pentapeptide) as precursors, the incorporation of **peptidoglycan** into the pre-existing cell wall of G. homari was inhibited to an extent of 50% (ID50 value) at a concentration of 0.25 .mu.g of penicillin G/ml. With **UDP**-GlcNAc and **UDP**-MurNAc-tetrapeptide as precursors the ID50 value was about 2500-fold greater (630 .mu.g/ml). The inhibition by penicillin G of the incorporation of **peptidoglycan** from **UDP** -MurNAc-[14C]Lys-pentapeptide could be overcome by addition of non-radioactive **UDP**-MurNAc-tetrapeptide to the incubation mixture. In the presence of 5 .mu.g of penicillin G/ml the incorporation of **peptidoglycan** formed from the mixture of **UDP**-MurNAc-Ala-DGlu-Lys-D[14C]Ala-D[14C]Ala and non-radioactive **UDP**-MurNAc-tetrapeptide proceeded virtually without release of D-[14C]alanine by transpeptidase activity. The enzyme preparation also exhibited DD-carboxypeptidase activity which was only slightly more sensitive to penicillin G and mecillinam than was the incorporation of **peptidoglycan** into the cell wall. Since the ID50 values for the .beta.-lactam antibiotics are similar to the concentrations required to inhibit the growth of G. homari to an extent of 50%, the DD-carboxypeptidase must be the killing site of both penicillin G and mecillinam.

L22 ANSWER 13 OF 13 MEDLINE
ACCESSION NUMBER: 75211115 MEDLINE
DOCUMENT NUMBER: 75211115
TITLE: D-Alanine-requiring cell wall mutant of Escherichia coli.
AUTHOR: Olden K; Ito S; Wilson T H
SOURCE: JOURNAL OF BACTERIOLOGY, (1975 Jun) 122 (3) 1310-21. Journal code: HH3. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197512

AB A mutant of Escherichia coli is described whose cells show a spherical or irregular morphology, associated with leakage of beta-galactosidase and other intracellular proteins. The expression of the morphologic abnormality is most marked when the mutant is grown in rich media and is suppressed by D-alamine, D-serine, D-glutamate, or glycine supplementation. D-Alanine is the most effective amino acid supplement, half maximally supressing this anomalous property at a concentration of 75 mug/ml, as measured by the reduction in beta-galactosidase released from the cells. The mutant is more sensitive to penicillin G, D-methionine, and D-valine

and it is relatively resistant to lysozyme. These phenotypic abnormalities are likewise corrected by the above supplementations. The relative rates of **peptidoglycan** synthesis in mutant and parent, grown under restrictive conditions, were measured both in vivo and in vitro by rates of incorporation of L-[14-D]alanine and **uridine-5'-diphosphate** -N-acetyl-D-[1-15C-A1-glucosamine, respectively. There is not metabolic block in the biosynthesis of **uridine-5** **'-diphosphate-N-acetyl-muramyl-** **pentapeptide** as shown by enzymic analysis and the lack of accumulation of **uridine-5'-diphosphate** -N-acetylmuramyl-peptide precursors. These preliminary studies suggest that the mutant possesses a defect in the biosynthesis of **peptidoglycan** although the exact lesion has not yet been established.

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